SUBSTITUTE SHEET (RULE 26)

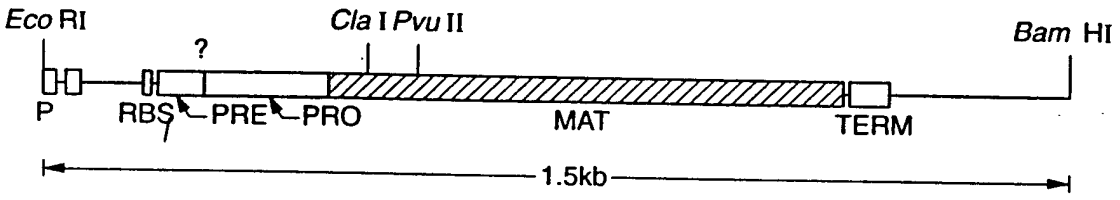


FIG._1A

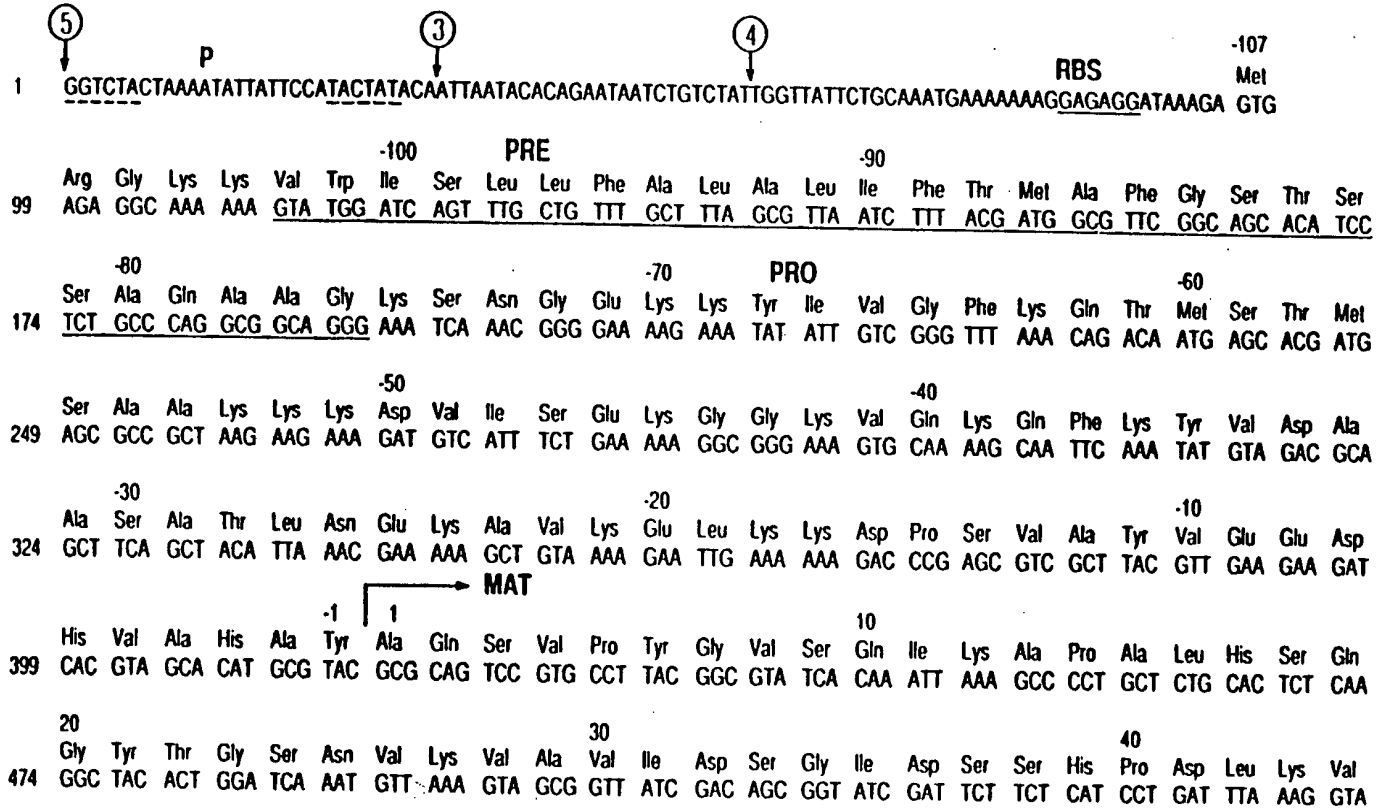

⑤
P
③
④
RBS
-107
Met
1 GGTCTACTAAAATATTATTCCATACTATACAATTAATACACAGAATAATCTGTCTATTGGTTATTCTGCAAATGAAAAAAAGGAGAGGATAAAGA GTG
PRE
-100
-90
Arg Gly Lys Lys Val Trp Ile Ser Leu Leu Phe Ala Leu Ala Leu Ile Phe Thr Met Ala Phe Gly Ser Thr Ser
99 AGA GGC AAA AAA GTA TGG ATC AGT TTG CTG TTT GCT TTA GCG TTA ATC TTT ACG ATG GCG TTC GGC AGC ACA TCC
-80
-70
PRO
-60
Ser Ala Gln Ala Ala Gly Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met Ser Thr Met
174 TCT GCC CAG GCG GCA GGG AAA TCA AAC GGG GAA AAG AAA TAT ATT GTC GGG TTT AAA CAG ACA ATG AGC ACG ATG
-50
-40
Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala
249 AGC GCC GCT AAG AAG AAA GAT GTC ATT TCT GAA AAA GGC GGG AAA GTG CAA AAG CAA TTC AAA TAT GTA GAC GCA
-30
-20
-10
Ala Ser Ala Thr Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala Tyr Val Glu Glu Asp
324 GCT TCA GCT ACA TTA AAC GAA AAA GCT GTA AAA GAA TTG AAA AAA GAC CCG AGC GTC GCT TAC GTT GAA GAA GAT
MAT
-1
1
10
His Val Ala His Ala Tyr Ala Gln Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln
399 CAC GTA GCA CAT GCG TAC GCG CAG TCC GTG CCT TAC GGC GTA TCA CAA ATT AAA GCC CCT GCT CTG CAC TCT CAA
20
30
40
Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val
474 GGC TAC ACT GGA TCA AAT GTT AAA GTA GCG GTT ATC GAC AGC GGT ATC GAT TCT TCT CAT CCT GAT TTA AAG GTA

FIG._1B - 1

```
                                                
```

```
                                        250 Gln                                                 260
    Gln  Val  Arg  Ser  Ser  Leu  Glu  Asn  Thr  Thr  Thr  Lys  Leu  Gly  Asp  Ser  Phe  Tyr  Tyr  Gly  Lys  Gly  Leu  Ile  Asn
1149 CAA  GTC  CGC  AGC  AGT  TTA  GAA  AAC  ACC  ACT  ACA  AAA  CTT  GGT  GAT  TCT  TTC  TAC  TAT  GGA  AAA  GGG  CTG  ATC  AAC

    270                       275
    Val  Gln  Ala  Ala  Ala  Gln  OC                    TERM
1224 GTA  CAG  GCG  GCA  GCT  CAG  TAA  AACATAAAAAACCGGCCTTGGCCCCGCCGGTTTTTTATTTTTCTTCCTCCGCATGTTCAATCCGCTCC

1316 ATAATCGACGGATGGCTCCCTCTGAAAATTTTAACGAGAAACGGCGGGTTGACCCGGCTCAGTCCCGTAACGGCCAAGTCCTGAAACGTCTCAATCGCCG

1416 CTTCCCGGTTTCCGGTCAGCTCAATGCCGTAACGGTCGGCGGCGTTTTCCTGATACCGGGAGACGGCATTCGTAATCGGATC
```

FIG._1B - 3

FIG._1B

- FIG._1B - 1
- FIG._1B - 2
- FIG._1B - 3

CONSERVED RESIDUES IN SUBTILISINS FROM *BACILLUS AMYLOLIQUEFACIENS*

```
1                   10                  20
A Q S V P . G . . . . . A P A . H . . G

21                  30                  40
. T G S . V K V A V . D . G . . . . H P

41                  50                  60
D L . . . G G A S . V P . . . . . Q D

61                  70                  80
. N . H G T H V A G T . A A L N N S I G

81                  90                  100
V L G V A P S A . L Y A V K V L G A . G

101                 110                 120
S G . . S . L . . G . E W A . N . . . .

121                 130                 140
V . N . S L G . P S . S . . . . . A . .

141                 150                 160
. . . . . G V . V V A A . G N . G . . .

161                 170                 180
. . . . . Y P . . Y . . . . A V G A .

181                 190                 200
D . . N . . A S F S . . G . . L D . . A

201                 210                 220
P G V . . Q S T . P G . . Y . . . N G T

221                 230                 240
S M A . P H V A G A A A L . . . K . . .

241                 250                 260
W . . . Q . R . . L . N T . . . L G . .

261                 270
. . Y G . G L . N . . A A . .
```

FIG._2

SUBSTITUTE SHEET (RULE 26)

COMPARISON OF SUBTILISIN SEQUENCES FROM:
B.amyloliquefaciens
B.subtilis
B.licheniformis
B.lentus

```
01                  10                  20                  30
A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T * H P

41                  50                  60                  70
D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G
D L N V V G G A S F V A G E A Y N * T D G N G H G T H V A G T V A A L D N T T G
D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G

81                  90                  100                 110
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D
V L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T T N G M D
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H

121                 130                 140                 150
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V V A A A G N E G T S G
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V A A A A G N E G S S G
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V V A A A G N S G N S G
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S
```

FIG._3A

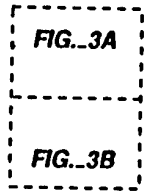
```
161                 170                 180                 190
S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
S T S T V G Y P A K Y P S T I A V G A V N S S N Q R A S F S S A G S E L D V M A
S T N T I G Y P A K Y D S V I A V G A V D S N S N R A S F S S V G A E L E V M A
* * * * I S Y P A R Y A N A M A V G A T D Q N N N R A S F S Q Y G A G L D I V A

201                 210                 220                 230
P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N
P G V S I Q S T L P G G T Y G A Y N G T S M A T P H V A G A A A L I L S K H P T
P G A G V Y S T Y P T N T Y A T L N G T S M A S P H V A G A A A L I L S K H P N
P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S

241                 250                 260                 270
W T N T Q V R S S L E N T T T K L G D S F Y Y G K G L I N V Q A A A Q
W T N A Q V R D R L E S T A T Y L G N S F Y Y G K G L I N V Q A A A Q
L S A S Q V R N R L S S T A T Y L G S S F Y Y G K G L I N V E A A A Q
W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R
```

FIG._3B

FIG._3



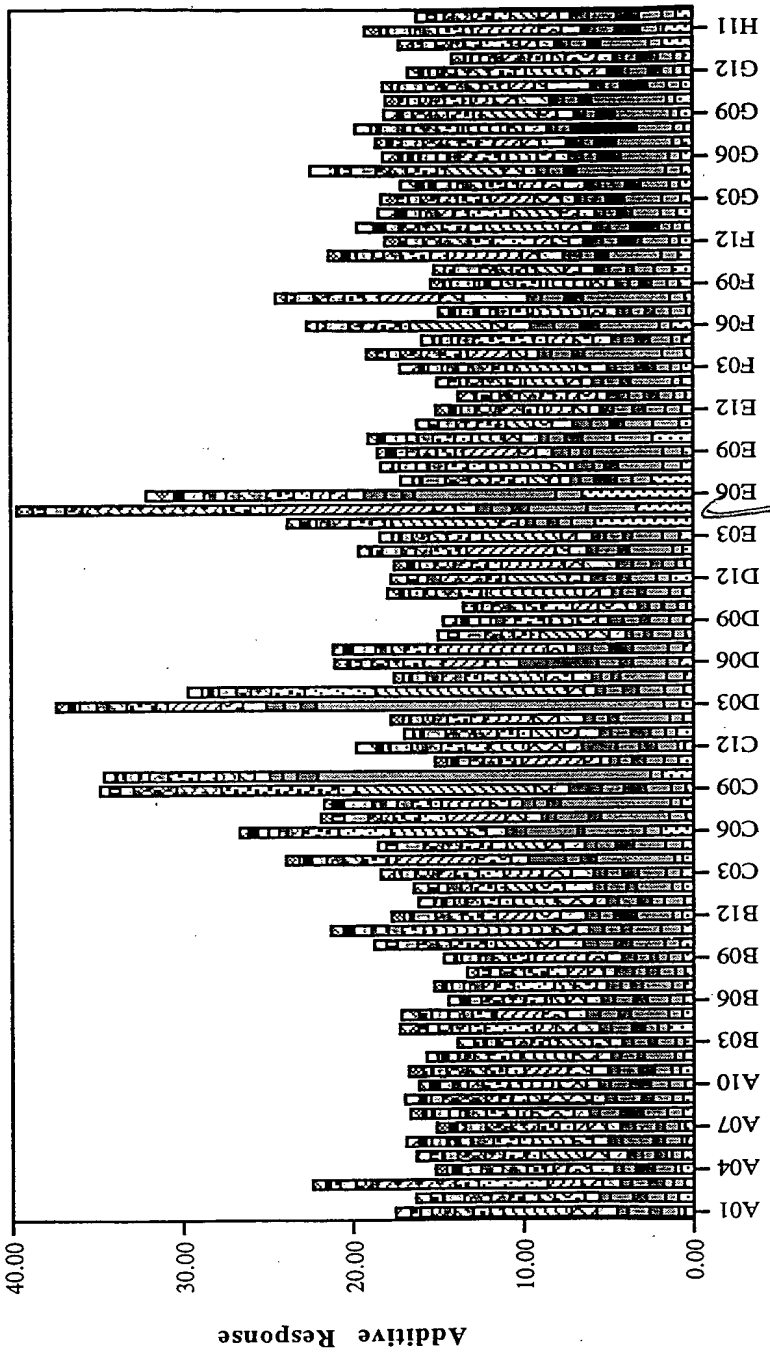

Additive Response
40.00
30.00
20.00
10.00
0.00
A01
A04
A07
A10
B03
B06
B09
B12
C03
C06
C09
C12
D03
D06
D09
D12
E03
E06
E09
E12
F03
F06
F09
F12
G03
G06
G09
G12
H11

FIG. 4

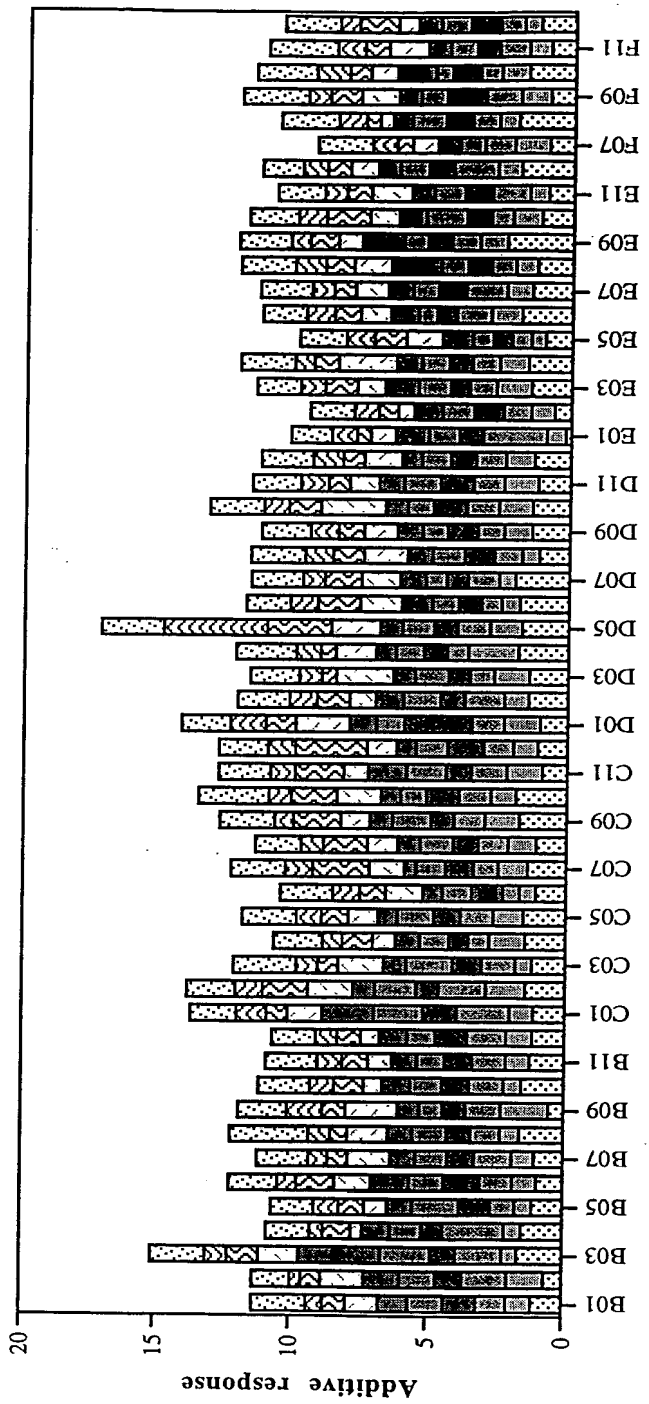


FIG. 5

09/06/2006, EAST Version: 2.0.3.0

1	A12	IKDFHVYFRESRDAG
2	A11	LEQAVNSATSRGVLV
3	A10	AQSVPWGISRVQAPA
4	A9	VPWGISRVQAPAAHN
5	A8	GISRVQAPAAHNRGL
6	A7	RVQAPAAHNRGLTGS
7	A6	APAAHNRGLTGSGVK
8	A5	AHNRGLTGSGVKVAV
9	A4	RGLTGSGVKVAVLDT
10	A3	TGSGVKVAVLDTGIS
11	A2	GVKVAVLDTGISTHP
12	A1	VAVLDTGISTHPDLN
13	B12	LDTGISTHPDLNIRG
14	B11	GISTHPDLNIRGGAS
15	B10	THPDLNIRGGASFVP
16	B9	DLNIRGGASFVPGEP
17	B8	IRGGASFVPGEPSTQ
18	B7	GASFVPGEPSTQDGN
19	B6	FVPGEPSTQDGNGHG
20	B5	GEPSTQDGNGHGTHV
21	B4	STQDGNGHGTHVAGT
22	B3	DGNGHGTHVAGTIAA
23	B2	GHGTHVAGTIAALNN
24	B1	THVAGTIAALNNSIG
25	C12	AGTIAALNNSIGVLG
26	C11	IAALNNSIGVLGVAP
27	C10	LNNSIGVLGVAPSAE
28	C9	SIGVLGVAPSAELYA
29	C8	VLGVAPSAELYAVKV
30	C7	VAPSAELYAVKVLGA
31	C6	SAELYAVKVLGASGS
32	C5	LYAVKVLGASGSGSV
33	C4	VKVLGASGSGSVSSI
34	C3	LGASGSGSVSSIAQG
35	C2	SGSGSVSSIAQGLEW
36	C1	GSVSSIAQGLEWAGN
37	D12	SSIAQGLEWAGNNGM
38	D11	AQGLEWAGNNGMHVA
39	D10	LEWAGNNGMHVANLS
40	D9	AGNNGMHVANLSLGS
41	D8	NGMHVANLSLGSPSP
42	D7	HVANLSLGSPSPSAT
43	D6	NLSLGSPSPSATLEQ
44	D5	LGSPSPSATLEQAVN
45	D4	PSPSATLEQAVNSAT
46	D3	SATLEQAVNSATSRG
47	D2	LEQAVNSATSRGVLV
48	D1	AVNSATSRGVLVVAA
49	E12	SATSRGVLVVAASGN
50	E11	SRGVLVVAASGNSGA
51	E10	VLVVAASGNSGAGSI
52	E9	VAASGNSGAGSISYP
53	E8	SGNSGAGSISYPARY
54	E7	SGAGSISYPARYANA
55	E6	GSISYPARYANAMAV
56	E5	SYPARYANAMAVGAT
57	E4	ARYANAMAVGATDQN
58	E3	ANAMAVGATDQNNNR
59	E2	MAVGATDQNNNRASF
60	E1	GATDQNNNRASFSQY
61	F12	DQNNNRASFSQYGAG
62	F11	NNRASFSQYGAGLDI
63	F10	ASFSQYGAGLDIVAP
64	F9	SQYGAGLDIVAPGVN
65	F8	GAGLDIVAPGVNVQS
66	F7	LDIVAPGVNVQSTYP
67	F6	VAPGVNVQSTYPGST
68	F5	GVNVQSTYPGSTYAS
69	F4	VQSTYPGSTYASLNG
70	F3	TYPGSTYASLNGTSM
71	F2	GSTYASLNGTSMATP
72	F1	YASLNGTSMATPHVA
73	G12	LNGTSMATPHVAGAA
74	G11	TSMATPHVAGAAALV
75	G10	ATPHVAGAAALVKQK
76	G9	HVAGAAALVKQKNPS
77	G8	GAAALVKQKNPSWSN
78	G7	ALVKQKNPSWSNVQI
79	G6	KQKNPSWSNVQIRNH
80	G5	NPSWSNVQIRNHLKN
81	G4	WSNVQIRNHLKNTAT
82	G3	VQIRNHLKNTATSLG
83	G2	RNHLKNTATSLGSTN
84	G1	LKNTATSLGSTNLYG
85	H12	TATSLGSTNLYGSGL
86	H11	SLGSTNLYGSGLVNA
87	H10	STNLYGSGLVNAEAA
88	H9	NLYGSGLVNAEAATR

FIG. 6A

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1	A12	IKDFHVYFRESRDAG
2	A11	DAELHIFRVFTNNQV
3	A10	PLRRASLSLGSGFWH
4	A9	RASLSLGSGFWHATG
5	A8	LSLGSGFWHATGRHS
6	A7	GSGFWHATGRHSSRR
7	A6	FWHATGRHSSRRLLR
8	A5	ATGRHSSRRLLRAIP
9	A4	RHSSRRLLRAIPRQV
10	A3	SRRLLRAIPRQVAQT
11	A2	LLRAIPRQVAQTLQA
12	A1	AIPRQVAQTLQADVL
13	B12	RQVAQTLQADVLWQM
14	B11	AQTLQADVLWQMGYT
15	B10	LQADVLWQMGYTGAN
16	B9	DVLWQMGYTGANVRV
17	B8	WQMGYTGANVRVAVF
18	B7	GYTGANVRVAVFDTG
19	B6	GANVRVAVFDTGLSE
20	B5	VRVAVFDTGLSEKHP
21	B4	AVFDTGLSEKHPHFK
22	B3	DTGLSEKHPHFKNVK
23	B2	LSEKHPHFKNVKERT
24	B1	KHPHFKNVKERTNWT
25	C12	HFKNVKERTNWTNER
26	C11	NVKERTNWTNERTLD
27	C10	ERTNWTNERTLDDGL
28	C9	NWTNERTLDDGLGHG
29	C8	NERTLDDGLGHGTFV
30	C7	TLDDGLGHGTFVAGV
31	C6	DGLGHGTFVAGVIAS
32	C5	GHGTFVAGVIASMRE
33	C4	TFVAGVIASMRECQG
34	C3	AGVIASMRECQGFAP
35	C2	IASMRECQGFAPDAE
36	C1	MRECQGFAPDAELHI
37	D12	CQGFAPDAELHIFRV
38	D11	FAPDAELHIFRVFTN
39	D10	DAELHIFRVFTNNQV
40	D9	LHIFRVFTNNQVSYT
41	D8	FRVFTNNQVSYTSWF
42	D7	FTNNQVSYTSWFLDA
43	D6	NQVSYTSWFLDAFNY
44	D5	SYTSWFLDAFNYAIL
45	D4	SWFLDAFNYAILKKI
46	D3	LDAFNYAILKKIDVL
47	D2	FNYAILKKIDVLNLS
48	D1	AILKKIDVLNLSIGG
49	E12	KKIDVLNLSIGGPDF
50	E11	DVLNLSIGGPDFMDH
51	E10	NLSIGGPDFMDHPFV
52	E9	IGGPDFMDHPFVDKV
53	E8	PDFMDHPFVDKVWEL
54	E7	MDHPFVDKVWELTAN
55	E6	PFVDKVWELTANNVI
56	E5	DKVWELTANNVIMVS
57	E4	WELTANNVIMVSAIG
58	E3	TANNVIMVSAIGNDG
59	E2	NVIMVSAIGNDGPLY
60	E1	MVSAIGNDGPLYGTI
61	F12	AIGNDGPLYGTLNNP
62	F11	NDGPLYGTLNNPADQ
63	F10	PLYGTLNNPADQMDV
64	F9	GTLNNPADQMDVIGV
65	F8	NNPADQMDVIGVGGI
66	F7	ADQMDVIGVGGIDFE
67	F6	MDVIGVGGIDFEDNI
68	F5	IGVGGIDFEDNIARF
69	F4	GGIDFEDNIARFSSR
70	F3	DFEDNIARFSSRGMT
71	F2	DNIARFSSRGMTTWE
72	F1	ARFSSRGMTTWELPG
73	G12	SSRGMTTWELPGGYG
74	G11	GMTTWELPGGYGRMK
75	G10	TWELPGGYGRMKPDI
76	G9	LPGGYGRMKPDIVTY
77	G8	GYGRMKPDIVTYGAG
78	G7	RMKPDIVTYGAGVRG
79	G6	PDIVTYGAGVRGSGV
80	G5	VTYGAGVRGSGVKGG
81	G4	GAGVRGSGVKGGCRA
82	G3	VRGSGVKGGCRALSG
83	G2	SGVKGGCRALSGTSV
84	G1	KGGCRALSGTSVASP
85	H12	CRALSGTSVASPVVA
86	H11	LSGTSVASPVVAGAV
87	H10	TSVASPVVAGAVTLL
88	H9	ASPVVAGAVTLLVST
89	H8	VVAGAVTLLVSTVQK
90	H7	GAVTLLVSTVQKREL
91	H6	TLLVSTVQKRELVNP
92	H5	VSTVQKRELVNPASM
93	H4	VQKRELVNPASMKQA
94	H3	RELVNPASMKQALIA
95	H2	VNPASMKQALIASAR
96	H1	ASMKQALIASARRLP

09/09/02 MON 08:47 [TX/RX NO 6003]

FIG. 6B

97	I12	IKDFHVYFRESRDAG
98	I11	DAELHIFRVFTNNQV
99	I10	KQALIASARRLPGVN
100	I9	LIASARRLPGVNMFE
101	I8	SARRLPGVNMFEQGH
102	I7	RLPGVNMFEQGHGKL
103	I6	GVNMFEQGHGKLDLL
104	I5	MFEQGHGKLDLLRAY
105	I4	QGHGKLDLLRAYQIL
106	I3	GKLDLLRAYQILNSY
107	I2	DLLRAYQILNSYKPQ
108	I1	RAYQILNSYKPQASL
109	J12	QILNSYKPQASLSPS
110	J11	NSYKPQASLSPSYID
111	J10	KPQASLSPSYIDLTE
112	J9	ASLSPSYIDLTECPY
113	J8	SPSYIDLTECPYMWP
114	J7	YIDLTECPYMWPYCS
115	J6	LTECPYMWPYCSQPI
116	J5	CPYMWPYCSQPIYYG

FIG. 6C

09608N2 .01E50

MKLVNIWLLLLVVLLCGKKHLGDRLEKKSFEKAPCPGCSHLTLKVEFSSTVVEYEYIVAFNGYFT
AKARNSFISSALKSSEVDNWRIIPRNNPSSDYPSDFEVIQIKEKQKAGLLTLEDHPNIKRVTPQR
KVFRSLKYAESDPTVPCNETRWSQKWQSSRPLRRASLSLGSGFWHATGRHSSRRLLRAIPRQVAQ
TLQADVLWQMGYTGANVRVAVFDTGLSEKHPHFKNVKERTNWTNERTLDDGLGHGTFVAGVIASM
RECQGFAPDAELHIFRVFTNNQVSYTSWFLDAFNYAILKKIDVLNLSIGGPDFMDHPFVDKVWEL
TANNVIMVSAIGNDGPLYGTLNNPADQMDVIGVGGIDFEDNIARFSSRGMTTWELPGGYGRMKPD
IVTYGAGVRGSGVKGGCRALSGTSVASPVVAGAVTLLVSTVQKRELVNPASMKQALIASARRLPG
VNMFEQGHGKLDLLRAYQILNSYKPQASLSPSYIDLTECPYMWPYCSQPIYYGGMPTVVNVTILN
GMGVTGRIVDKPDWQPYLPQNGDNIEVAFSYSSVLWPWSGYLAISISVTKKAASWEGIAQGHVMI
TVASPAETESKNGAEQTSTVKLPIKVKIIPTPPRSKRVLWDQYHNLRYPPGYFPRDNLRMKNDPL
DWNGDHIHTNFRDMYQHLRSMGYFVEVLGAPFTCFDASQYGTLLMVDSEEEYFPEEIAKLRRDVD
NGLSLVIFSDWYNTSVMRKVKFYDENTRQWWMPDTGGANIPALNELLSVWNMGFSDGLYEGEFTL
ANHDMYYASGCSIAKFPEDGVVITQTFKDQGLEVLKQETAVVENVPILGLYQIPAEGGGRIVLYG
DSNCLDDSHRQKDCFWLLDALLQYTSYGVTPPSLSHSGNRQRPPSGAGSVTPERMEGNHLHRYSK
VLEAHLGDPKPRPLPACPRLSWAKPQPLNETAPSNLWKHQKLLSIDLDKVVLPNFRSNRPQVRPL
SPGESGAWDIPGGIMPGRYNQEVGQTIPVFAFLGAMVVLAFFVVQINKAKSRPKRRKPRVKRPQL
MQQVHPPKTPSV

FIG. 7

09/508,376 - 371 Date

WO 95/23857 PCT/US95/02454

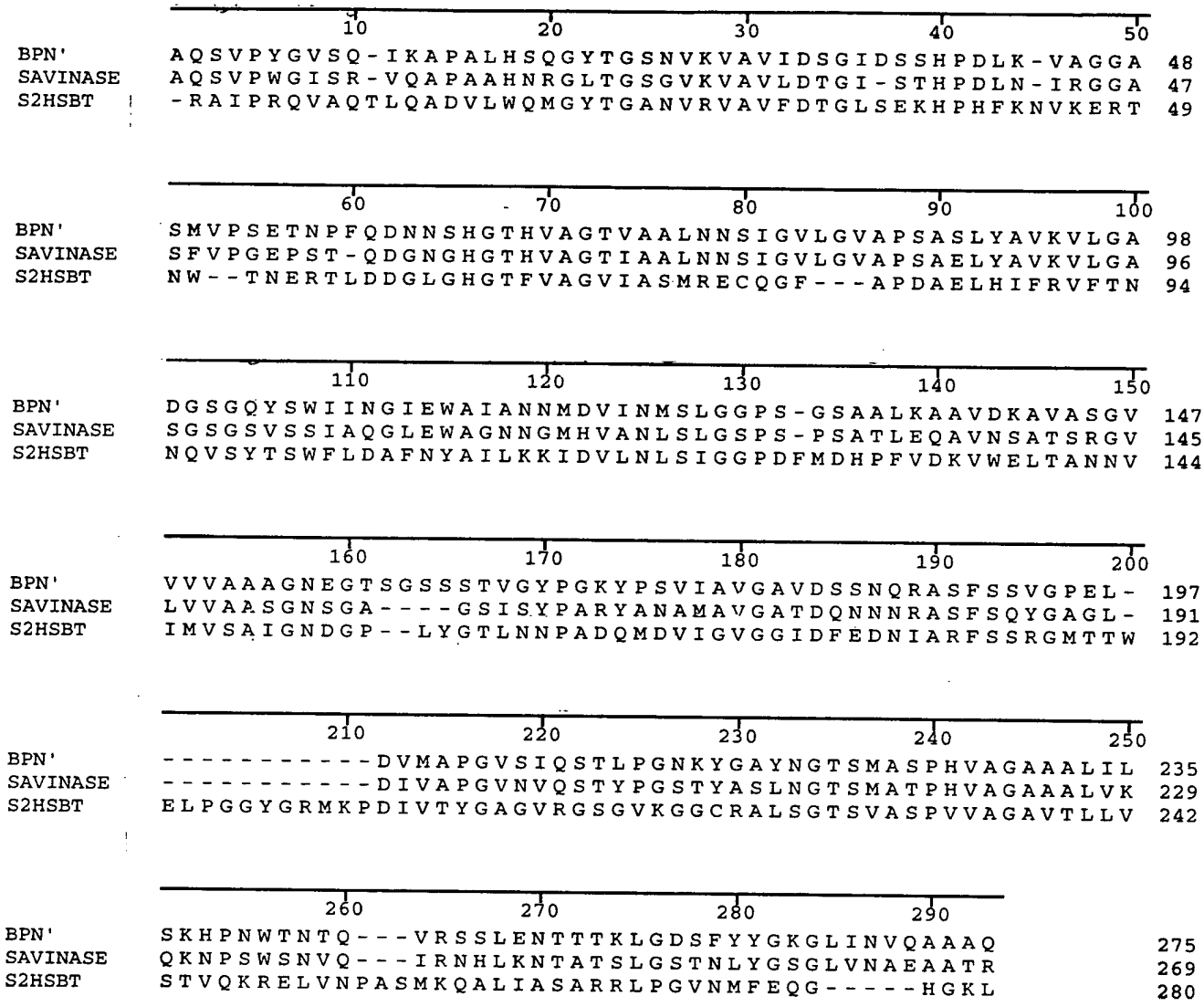

FIG. 8

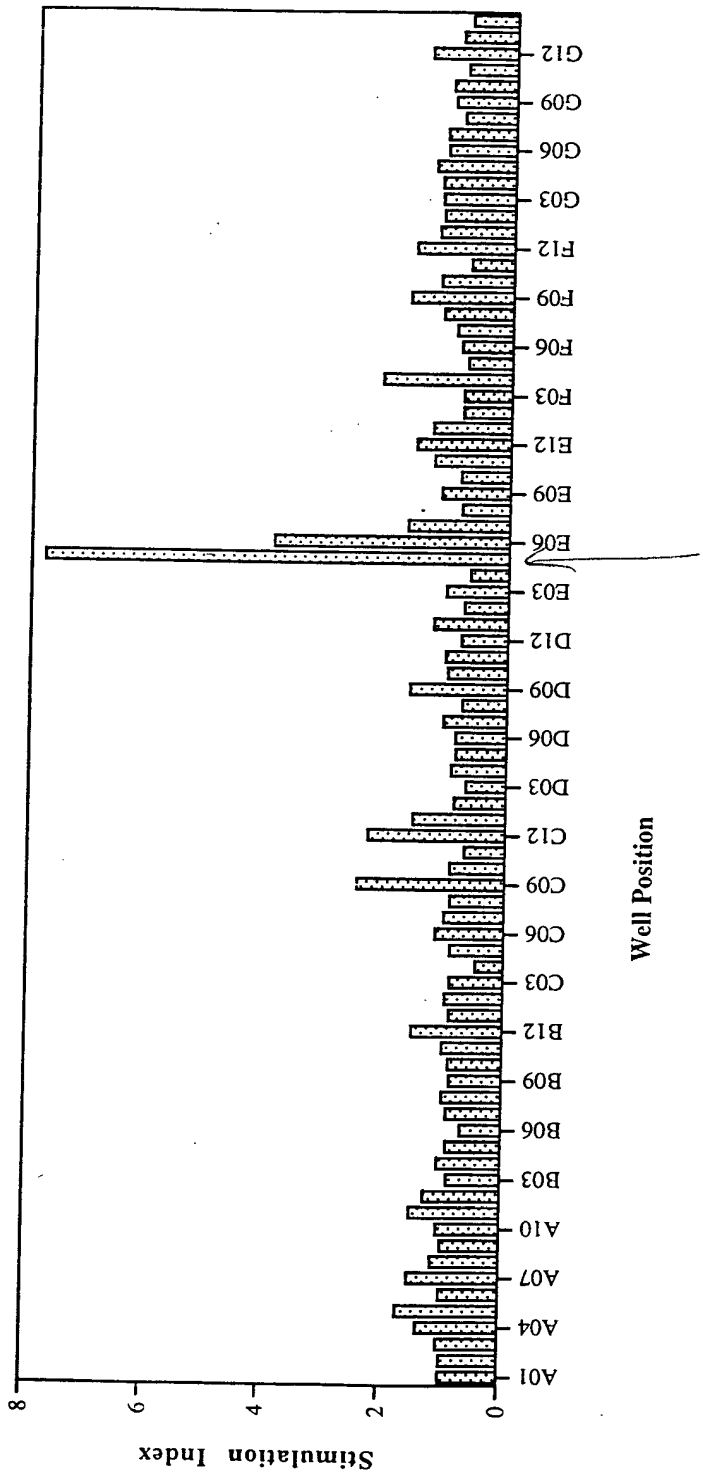

FIG. 9

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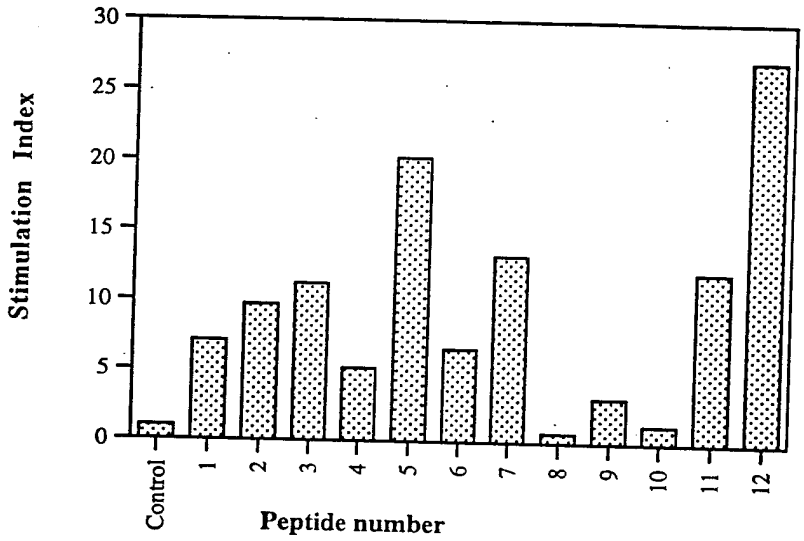

FIG. 10

WO 96/27604 PCT/US96/02959